## REMARKS

The present invention relates to a method of inducing and enhancing the proliferation of human marrow stromal cells.

Claims 1 through 41 are pending in the present application. Claims 13, 30 and 37-41 have been canceled herein without prejudice to the inclusion of the subject matter contained therein in any later filed continuation and/or divisional application(s) as being drawn to non-elected inventions. Therefore, claims 1-12, 14-29 and 31-36 are currently under examination following entry of the present Amendment.

Applicants respectfully thank Examiner Ram R. Shukla, Ph.D. for participating in a telephone interview dated February 26, 2004 with the undersigned, Kathryn Doyle, Ph.D., J.D. Per the telephone conference, wherein the undersigned and Examiner Ram R. Shukla discussed amending the claims to recite replating at a density less than 50 cells/$cm^2$, the Examiner indicated that the he would consider such amendments to the claims. Accordingly, claims 1, 24, 31 and 32 have been amended herein to incorporate a replating step wherein the isolated marrow stromal cells are replated at least one time at a density of less than about 50 cells per square centimeter. Support for the amendments to claims 1, 24, 31 and 32 is found throughout the as-filed specification as fully set forth below. As such, no new matter has been added by way of the present Amendment.

Further, claims 12 and 36 have been amended herein to more particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Specifically, claim 12 has been amended to properly depend from claim 1, and claim 36 has been amended to clarify the reference to Figure 2. No new matter has been added by way of these amendments.

Rejection of Claims 1-21 pursuant to 35 U.S.C. §102(b)

The Examiner has maintained his rejection of claims 1-21 under 35 U.S.C. §102(b). Specifically, the Examiner asserts that Bruder et al. (1997, Journal of Cellular Biochemistry 64: 278-294; Bruder) teaches isolated bone marrow cells that have approximately 1 marrow stromal cell in a population of $10^5$ cells, which therefore represents 100 cells in a 60 $cm^2$ dish. The Examiner is of the opinion that Bruder meets the limitations of the claimed invention. Applicants respectfully submit that Bruder does not anticipate the present invention for the

following reasons.

It is hornbook law that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP §2131 (quoting *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). "The identical invention must be shown in as complete detail as is contained in the . . . claim." *Id.* (quoting *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989) (emphasis added). Therefore, Bruder must describe each and every element of claims 1-21, in order to anticipate these claims under 35 U.S.C. §102(b), and this reference does not.

Applicants respectfully point out that Bruder describes that the hMSC-enriched low density fraction was initially plating at $10^7$ nucleated cells per 60 $cm^2$, and Bruder estimates that the initial density of adherent hMSCs is approximately 1 in $10^5$ of the total cell population, which therefore would correspond to 100 adherent hMSC in a 60 $cm^2$ dish. However, Applicants also respectfully point out that Bruder further teaches culturing the initial adherent hMSC population until the cells are confluent, and then replating the cells at a density of 3 X $10^3$ cells per $cm^2$. Specifically, Figure 1 in Bruder teaches that the lowest density of adherent hMSCs in the replating step and the subsequent serial passages to encompass replating the cells at a density of 3 X $10^3$ cells per $cm^2$. It is Applicants' view that a density of 3 X $10^3$ cells per $cm^2$ is not less than about 50 cells per $cm^2$, as recited in claim 1 and dependents claims therefrom following entry of the present Amendment. That is, claims 1, 24, 31 and 32 have been amended herein to recite a replating step wherein the isolated human marrow stromal cells are replated at a density of less than about 50 cells per $cm^2$. Support for expanding and replating human marrow stromal cells is found throughout the specification. For example, beginning on line 25 of page 19, the specification discloses that the expansion of MSCs should not encompass plating the cells at a density of greater than about 50 cells per $cm^2$. Further, beginning on line 6 of page 22, the specification teaches the replating of MSCs wherein the expanded MSCs are replated to a second growth surface at a density of less than about 50 per $cm^2$. Therefore, in view of the present amendments to the claims, Bruder cannot anticipate the present invention because Bruder does not disclose each and every element of the claimed invention. Reconsideration and withdrawal of the Examiner's rejection pursuant to 35 U.S.C. §102(b) is respectfully requested at this time.

Rejection of Claims 1-21 pursuant to 35 U.S.C. §102(b)

The Examiner has maintained his rejection of Claims 1-21 under 35 U.S.C. §102(b) over Kuznetsov et al. (Journal of Bone and Mineral Research 12: 1335-1347; Kuznetsov). Specifically, the Examiner contends that Kuznetsov teaches single colony derived strains of human marrow stromal fibroblasts (HMSFs) plated at 0.14-14 X $10^3$ cells/$cm^2$ or .007-3.5 X $10^3$ cells/$cm^2$, and that such teachings anticipate the present invention. Applicants respectfully submit that Kuznetsov does not anticipate the present invention for the following reasons.

The left column on page 1137 of Kuznetsov discloses that the primary cultures were grown to produce multicolony derived strains, wherein the cells produced a high number of HMSFs during the first passage. Subsequently, the cells were collected and plated at a density of 0.1-0.2 X $10^5$ cells per $cm^2$ and replated accordingly at a density of 0.1-0.2 X $10^5$ cells per $cm^2$. Plating and replating at a density of 0.1-0.2 X $10^5$ cells per $cm^2$ is not less than about 50 cells per $cm^2$, as recited in claim 1 and dependent claims therefrom. Therefore, Kuznetsov does not anticipate the present invention because Kuznetsov does not teach each and every element of the instant claims.

Applicants, in view of the foregoing arguments, respectfully request reconsideration and withdrawal of the rejection of claims 1-21 pursuant to 35 U.S.C. §102(b) as being anticipated by Kuznetsov.

Rejection of Claims 1 and 22-23 pursuant to 35 U.S.C. §103(a)

The Examiner has maintained his rejection of claims 1 and 22-23 under 35 U.S.C. §103(a) as being *prima facie* obvious over Kuznetsov in view of Azizi et al. (1998, Proc. Nat'l. Acad. Sci. USA 95: 3908-3913; Azizi). Specifically, the Examiner is of the opinion that Kuznetsov teaches single colony derived strains of human marrow stromal fibroblasts (HMSFs) plated at 0.14-14 X $10^3$ cells/$cm^2$ or .007-3.5 X $10^3$ cells/$cm^2$ and that Azizi teaches growing human marrow stromal cells in a medium comprising PDGF-AA. Therefore, the Examiner reasons that it would have been *prima facie* obvious to combine the teachings of Kuznetsov and Azizi to arrive at the present invention as recited in claim 1 and 22-23.

Applicants submit that Kuznetsov and Azizi cannot render claims 1 and 22-23 *prima facie* obvious under 35 U.S.C. §103(a). The deficiencies of Kuznetsov discussed above

and although they are not repeated here, they are equally applicable to the rejection of the claims under 35 U.S.C. §103(a). Azizi merely teaches the addition of PDGF-AA to the culture medium in order to increase in the growth rate of marrow stromal cells, and therefore the teachings of Azizi cannot correct the deficiencies of Kuznetsov. More specifically, the MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

The present invention encompasses a method of inducing and enhancing the proliferation of human marrow stromal cells *in vitro* by plating the isolated cells at very low densities in the presence of a growth medium, harvesting the cells, and providing the cells to a second growth surface at a certain density of cells in the presence of growth medium. As amply detailed in the specification, such as in Example 2, beginning at page 36, the methods presently claimed result in a dramatic increase in the number of population doublings of the cells in the absence of differentiation. Kuznetsov does not suggest or motivate the skilled artisan to arrive at the present invention. That is, Kuznetsov does not suggest that when isolated MSCs are plated at very low densities, MSCs are induced to rapidly proliferate and increase in number at levels unheralded in the prior art. Further, Kuznetsov does not suggest the replating of isolated MSCs at a very low density, for example at a density less than about 50 cells per $cm^2$.

Applicants contend that the teachings of Azizi do not correct the deficiencies of Kuznetsov. That is, Azizi teaches the isolation of human MSCs from bone marrow aspirate of normal male and female vounteers, with the initial isolation step comprising density gradient centrifugation, and the secondary isolation step comprising separating MSCs from other bone marrow cells by their adherence to a plastic substrate. Azizi teaches that bone marrow aspirates, before they are separated from non-adherent cells, are plated at a density of $3 \times 10^6$ cells per $cm^2$. After non-adherent cells are removed, the adherent cells are grown to confluence, split at a ratio of 1:2 or 1:3, and replated in the presence of PDGF-AA. Azizi suggests that the addition of PDGF-AA increases the growth rate of the cells, but there is no suggestion that decreasing the

initial density of isolated MSCs or decreasing the density of harvested MSCs provided to a second growth surface or replating the cells at a low density would further induce proliferation. In fact, the only suggestion made in order to increase the number of MSCs for the experiments described in Azizi is to add PDGF-AA to the media. There is no suggestion that decreasing the initial or secondary plating density would result in an increased proliferation of MSCs. Further, there would have been no motivation to combine Kuznetsov with Azizi to achieve the surprising results disclosed in the present application. Therefore, Kuznetsov when combined with Azizi does not render the present invention *prima facie* obvious.

The second criteria for establishing a *prima facie* case of obviousness is that there must be a reasonable expectation of success. Kuznetsov only describes the proliferative and morphology characteristics of MSCs plated at a density of 140 cells per $cm^2$. Kuznetsov does not describe replating cells at a density of less than about 50 cells per $cm^2$. Thus, as discussed elsewhere herein, the teachings set forth in Kuznetsov would not have provided a reason to expect an unexpected increase in the proliferation of cells plated at a density less than 140 cells per $cm^2$. In fact, Kuznetsov teaches away from the present invention. That is Kuznetsov teaches the plating of MSCs at low densities to prevent colony cross-contamination rather than for the enhanced proliferation of the cells. Therefore, Applicants contend that one of skill in the art would have no reason to expect success in inducing proliferation of MSCs by plating the cells at very low densities other than to prevent contamination, and therefore, Kuznetsov fails to render the present invention *prima facie* obvious under 35 U.S.C. §103(a).

Similarly, Kuznetsov in view of Azizi does not offer one of skill in the art a reasonable expectation of success to arrive at the unexpected proliferation rate using the methods of the present invention. That is, Azizi teaches the addition of PDGF-AA to the culture medium to increase the growth rate of the cells. Nowhere does Azizi teach replating cells at a density of less than about 50 cells per $cm^2$. Therefore, Applicants contend that the teachings of Azizi do not correct the deficiencies of Kuznetsov, and thus one of skilled in the art would have no reasonable expectation of success in combining the teaching of the two references to arrive at the present invention.

The third prong in establishing a *prima facie* case of obviousness requires the prior art reference or references to teach or suggest all of the claim limitations. As detailed elsewhere herein, Kuznetsov does not teach replating at a low density, for example less than

about 50 cells per $cm^2$ for inducing proliferation of the isolated human marrow stromal cells. Therefore, Kuznetsov does not teach or suggest all of the claim limitations. Further, the teachings of Azizi do not correct the deficiencies of Kuznetsov because as discussed elsewhere herein, Azizi merely teaches the use of PDGF-AA to increase the growth rate of the cells. Azizi does not teach replating cells at a density of less than about 50 cells per $cm^2$. Therefore, Kuznetsov in view of Azizi cannot render the present invention *prima facie* obvious under 35 U.S.C. §103(a).

Applicants respectfully submit that Kuznetsov in view of Azizi cannot render claims 1 and 22-23 *prima facie* obvious, and request reconsideration and withdrawal of the Examiner's rejection pursuant to 35 U.S.C. §103(a).

Rejection of claims 1 and 24-29 and 31-36 pursuant to 35 U.S.C. §103(a)

The Examiner has maintained his rejection of claims 1, 24-29 and 31-36 under 35 U.S.C. §103(a) as being unpatentable over Kuznetsov in view of Azizi and in further view of Greenberger et al. (U.S. Patent No 5,766,950; Greenberger) and Prockop (1997, Science 276: 71-74). Specifically, the Examiner is of the opinion that it would have been *prima facie* obvious for one of skill in the art to combine the culture conditions of Kuznetsov with the addition of PDGF-AA taught in Azizi as well as the conditioned medium taught by Greenberger and the general properties of MSCs taught by Prockop et al. to arrive at the present invention.

Applicants submit that Kuznetsov in view of Azizi and in further view of Greenberger and Prockop cannot render claims 1, 24-29 and 31-36 *prima facie* obvious under 35 U.S.C. §103(a). As discussed elsewhere herein, Kuznetsov in view of Azizi cannot render the present claims *prima facie* obvious and the teachings of Greenberger and Prockop cannot correct the deficiencies of Kuznetsov and Azizi. Specifically, Greenberger teaches culturing primary bone marrow cells at an initial total number of $2.75 \times 10^7$ cells and then growing the cells until confluency before replating. The cells are replated at a total number of $2.5 \times 10^6$ cells. Nowhere does Greenberger teach plating and replating at a low density to induce increased proliferation of isolated human marrow stromal cells. With respect to Prockop, Prockop merely provides a review of the history of MSCs and teaches the potential uses of MSCs, and therefore does not, in anyway, read on the present invention. That is, Prockop does not disclose the plating and replating at a low density to induce increased proliferation of MSCs. Therefore, the

combined teachings of Greenberger and Prockop do not correct the deficiencies of Kuznetsov and Azizi. Therefore, Applicants contend that Kuznetsov in view of Azizi and further in view of Greenberger and Prockop cannot render the present invention *prima facie* obvious under 35 U.S.C. §103(a).

Applicants, in view of the foregoing arguments, respectfully request reconsideration and withdrawal of the rejection of claims 1 and 24-29 and 31-36 pursuant to 35 U.S.C. §103(a) as being *prima facie* obvious.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that claims 1-12, 14-29 and 31-36 are now in condition for allowance. Applicants further submit that no new matter has been added by way of the present amendment. Reconsideration and allowance of these claims is respectfully requested at the earliest possible date.

Respectfully submitted,

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Enclosure: Petition for Extension of Time and fee therefor